HARMONY new harmonised protocol for RFLP analysis of *Sma*I–restricted chromosomal DNA of MRSA using pulsed-field gel electrophoresis (a modified version of the Belgian protocol).

Safety

All local safety procedures should be followed.

Gloves and safety spectacles should be worn throughout the procedure.

Ethidium bromide is teratogenic and should not be handled if pregnant.

Unless an enclosed gel documentation system is used, UV protective visors should be worn when visualising and photographing gels.

1. Method of DNA preparation

Prepare DNA blocks using your standard laboratory procedure. Perhaps the most important point to note here is the cell concentration of the bacterial suspension to be incorporated into the agarose block. In LHI (London), we prepare a suspension equivalent to a McFarland standard 3 (approx. 9×10^8 cells/ml) and mix equal volumes of a 2% low melting point (LMP) agarose to pour into the block moulds.

In ULB (Brussels), they prepare a cell suspension equivalent to 3-4 x 10^9 CFU/ml, then mix with an equal volume of LMA and pour into their ultra thin moulds.

2. Digestion

Digest blocks according to local protocol

Alternatively

2.1 Equilibrate 2-3 mm portion of block for each isolate in *Sma*I buffer at 4^{o}C for 15-30 minutes prior to digestion.

2.2 Cover with 100µl of *Sma*I reaction buffer containing 20U of *Sma*I restriction enzyme (10U for plugs from Belgian Ultra-thin moulds). Mix gently and incubate at the recommended temperature (depends on manufacturer, usually 25 or 30^{o}C) for at least 4 hours.

3. PFGE

3.1 Pre-chill 1700 ml of 0.5 x TBE.

3.2 Prepare 150 ml of a 1% agarose gel using molecular grade agarose in 0.5 x TBE. Clean gel-casting frame (20.5cm x 14cm) and well comb with alcohol and wipe with distilled water to remove any dust immediately prior to pouring (dust/paper fluoresces!). Use an appropriate positioning frame for optimum results. Pour gel once agarose cools to 60^{o}C.

3.3 For lambda from BioRad we recommend heat-treating at 52^{o}C for 8 mins - place a 1mm-thick piece of lambda ladder in an 0.5ml reaction tube, cover with sufficient (i.e. not too much!) buffer (from stock lambda container) and incubate for 8 mins in a 52^{o}C waterbath. However, for inter-centre comparisons we recommend using NCTC 8325 as the reference standard due to the variable mobility of lambda concatemers observed with lambda from different suppliers.

3.4 Load digested plugs and molecular weight standards (NCTC 8325) into wells. Place standards to run in every 5th-6th lane. Seal wells with molten agarose (same as for gel) kept at 56^{o}C.

3.5 Place gel in electrophoresis tank, ensuring it is securely in place using plastic wedges and tape. Pour 1700 ml of pre-chilled electrophoresis buffer, 0.5 x TBE, into tank to cover the gel. Replace lid and check that apparatus is correctly connected and the tank is on a flat surface (i.e. no tubing or wires beneath any of the legs!) to ensure an even depth of buffer throughout the tank (to prevent differential cooling across the gel.)

3.6 Switch on circulating pump and check to make sure there are no bubbles in the tubing. Switch on chiller unit and set at 14^{o}C. (We have found that the actual temperature in the tank is up to 2^{o}C lower than that reported by the cooling module!) Allow temperature of gel and buffer to equilibrate for at least 30 minutes, if time permits.

3.7 Set the following parameters:

Block 1: initial switch time – 5 seconds; final switch time – 15 seconds; run time – 10 hours; voltage – 200 V or 6V/cm.

Block 2: initial switch time – 15 seconds; final switch time – 60 seconds; run time – 13 hours; voltage – 200 V or 6V/cm.

If using the CHEF Mapper system, the included angle is 120^{o} and the ramp factor is linear.

3.8 Start run.

4. Staining, visualising and photographing gel, and storing image.

4.1 Stain gel for 30-45 mins in ethidium bromide (1µg/ml). Wear gloves when handling and do not handle if pregnant as teratogenic.

4.2 De-stain for 45 mins in distilled water.

4.3 View under UV transillumination and photograph using gel documentation system, print and store image on a floppy disc as a .tif file for transfer to computer and GelCompar for analysis.